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Applicants: Jonathan S. Stamler and Andrew J. Gow
Application No.: 08/796,164 Group: 1639
Filed: February 6, 1997 Examiner: Bennett M. Celsa
Confirmation No.: 8622
For: Modified Hemoglobins, Including Nitrosylhemoglobins, and Uses Therefor

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REPLY

Commissioner for Patents
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Sir:

This Reply is in response to the Office Communication mailed from the United States Patent and Trademark Office on 30 June 2003. The Examiner has requested an appendix of the current pending claims in the present application. A list of the current pending claims, entitled "Claims as Amended with Supplemental Amendment April 12, 2001" is attached as Appendix A.

Respectfully submitted,

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Claims as Amended with Supplemental Amendment April 12, 2001

11. (Amended) A method for making SNO-Hb[FeII]O₂, which is specifically S-nitrosylated on thiol groups, comprising incubating excess nitrosating agent with purified hemoglobin in the presence of oxygen at pH 7.4 to 9.2.
12. The method of Claim 11 in which the nitrosating agent is a low molecular weight S-nitrosothiol.
14. (Amended) A method for making SNO-Hb[FeII], which is specifically S-nitrosylated on thiol groups, comprising incubating excess nitrosating agent with purified hemoglobin in the absence of oxygen at pH 7.4 to 9.2.
15. The method of Claim 14 in which the nitrosating agent is a low molecular weight S-nitrosothiol.
16. (Twice Amended) A method for potentiating delivery of NO to tissues in a mammal, comprising administering to the mammal an effective amount of a mixture of a low molecular weight thiol and hemoglobin or nitrosated hemoglobin.
17. (Amended) A method for delivering NO in a mammal, comprising administering to the mammal an effective amount of a blood substitute comprising nitrosated hemoglobin.

18. The method of Claim 17, in which the blood substitute comprises nitrosated hemoglobin and low molecular weight S-nitrosothiol.
19. (Amended) A method for scavenging oxygen free radicals and $\text{NO}\cdot$ in a mammal, comprising administering to the mammal an effective amount of a blood substitute comprising nitrosated hemoglobin.
20. (Amended) A method for reducing blood pressure in a mammal, comprising administering an effective amount of nitrosated hemoglobin to the mammal.
21. (Amended) A method for treating a disease in a mammal, comprising administering an effective amount of nitrosated or nitrated hemoglobin to the mammal, wherein the disease is selected from the group consisting of heart disease, brain disease, vascular disease, atherosclerosis, lung disease and inflammation.
22. (Amended) A method for treating a medical condition in a mammal, comprising administering an effective amount of nitrosated hemoglobin to the mammal, wherein the medical condition is selected from the group consisting of stroke, angina and acute respiratory distress.
27. A method for treating a patient having a disease or medical condition characterized by abnormalities of nitric oxide and oxygen metabolism, comprising administering to the patient an effective amount of a preparation comprising nitrosated hemoglobin.
28. The method of Claim 27 in which the disease or medical condition is selected from the group consisting of: heart disease, lung disease, sickle-cell anemia, stroke, sepsis or organ transplantation.

29. A blood substitute comprising nitrosated or nitrated hemoglobin.
40. (Amended) Method for delivering NO or its biological equivalent to tissues in an animal or human, comprising administering to the animal or human an effective amount of one or more nitrosyl-heme-containing donors of NO.
41. The method of Claim 40 wherein the nitrosyl-heme-containing donor of NO is nitrosylhemoglobin.
43. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin and buffer having a pK of at least about 9.4, at a concentration of approximately 10 mM to 200 mM, at pH 7.4.
44. Method for making SNO-oxyhemoglobin, comprising adding NO to an aqueous solution of oxyhemoglobin in a approximately 10 mM phosphate buffer at pH 7.4.
46. (Twice Amended) Method for making S-nitrosohemoglobin comprising adding NO to oxyhemoglobin in an aqueous solution at pH 7.4 to 9.2 such that the ratio of NO:hemoglobin is less than about 1:30.
63. (Twice Amended) A method for producing SNO-oxyhemoglobin, said method comprising mixing nitric oxide and deoxyhemoglobin at pH 7.4 and at a heme:NO ratio of less than 10, and exposing the resulting solution to air.
65. (Twice Amended) A method for producing S-nitrosohemoglobin, said method comprising mixing nitric oxide dissolved in an aqueous solution and purified oxyhemoglobin at a heme:NO ratio of less than about 10 in aqueous buffer at pH 7.4.

67. (Amended) A composition comprising S-nitrosylated oxyhemoglobin.
68. (Amended) A method for making a composition comprising S-nitrosylated oxyhemoglobin, comprising incubating excess nitrosating agent with purified hemoglobin in the presence of oxygen at pH 7.4 to 9.2.
69. The method of Claim 68 in which the nitrosating agent is a low molecular weight S-nitrosothiol.
70. (Amended) A composition comprising S-nitrosylated deoxyhemoglobin.
71. (Amended) A method for making a composition comprising S-nitrosylated deoxyhemoglobin, comprising incubating excess nitrosating agent with purified hemoglobin in the absence of oxygen at pH 7.4 to 9.2.
72. The method of Claim 71 in which the nitrosating agent is a low molecular weight S-nitrosothiol.
73. A method for reducing blood pressure in a mammal, comprising administering to the mammal an effective amount of a composition comprising nitrosylhemoglobin.
74. A method for treating a disease in a mammal, comprising administering an effective amount of a composition comprising nitrosylhemoglobin to the mammal, wherein the disease is selected from the group consisting of heart disease, brain disease, vascular disease, atherosclerosis, lung disease and inflammation.
75. A method for treating a medical condition in a mammal, comprising administering an effective amount of a composition comprising nitrosylhemoglobin to the

mammal, wherein the medical condition is selected from the group consisting of stroke, angina and acute respiratory distress.

76. A method for treating a patient having a disease or medical condition characterized by abnormalities of nitric oxide and oxygen metabolism, comprising administering to the patient an effective amount of a composition comprising nitrosylhemoglobin.
77. A method for producing a composition comprising SNO-methemoglobin, comprising incubating excess S-nitrosothiol with purified methemoglobin at pH 7.4 to 9.2.
78. A method for treating a disease in a mammal, comprising administering an effective amount of nitrosated hemoglobin to the mammal, wherein the disease is selected from the group consisting of heart disease, brain disease, vascular disease, atherosclerosis, lung disease and inflammation.
79. A blood substitute comprising nitrosated hemoglobin